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Modeling the Catalytic Cycle of Liver Alcohol Dehydrogenase: Synthesis and Structural Characterization of a Four-Coordinate Zinc Ethoxide Complex and Determination of Relative Zn−OR *versus* Zn−OH Bond Energies

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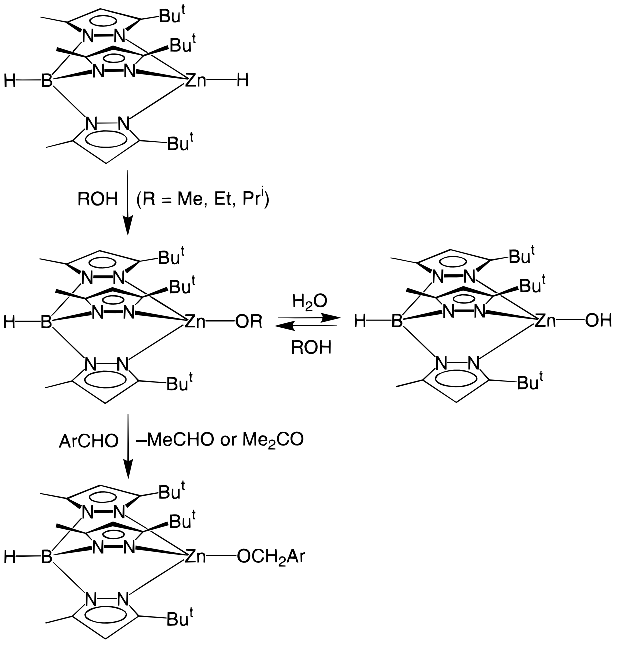
Liver alcohol dehydrogenase (LADH) belongs to a class of enzymes which catalyze the biological oxidation of alcohols to aldehydes and ketones.[1] The active site of the enzyme is composed of a distorted tetrahedral $\{[NSS]Zn(OH_2)\}^{2+}$ core in which the nitrogen and sulfur donors are provided by histidine and cysteine residues of a single polypeptide chain.[2] Two of the proposed critical steps by which the enzyme achieves the oxidation involve (i) the formation of a zinc alkoxide species and (ii) a formal "hydride" transfer from the alkoxide to the oxidized form of nicotinamide adenine dinucleotide (NAD^+). Consequently, it is important to isolate and structurally characterize such alkoxide complexes and investigate their potential for participating in alcohol oxidation. In this paper, we describe related chemistry of well-defined four-coordinate zinc hydroxide and alkoxide complexes which provides convincing evidence for both of the above transformations.

One of our current interests is concerned with developing the bioinorganic chemistry of zinc in an attempt to provide an understanding of the mechanism of action of enzymes such as carbonic anhydrase,[3−5] thermolysin,[6] bacteriophage T7 lysozyme,[7] bovine 5-aminolevulinate dehydratase,[7] peptide deformylase,[7] and liver alcohol dehydrogenase.[8] With respect to the latter enzyme, a four-coordinate zinc alkoxide species has been postulated as a critical intermediate of the catalytic cycle. However, despite their importance, there is little precedent for the formation of simple alkoxide derivatives from either zinc aqua or hydroxide derivatives. We therefore sought to determine whether a monomeric zinc hydroxide complex would be capable of generating a four-coordinate terminal alkoxide derivative from an unactivated alcohol such as ethanol.

While it would clearly be more appropriate to study a zinc hydroxide complex supported by a [NSS] coordination environment, the nonexistence of a well characterized [NSS]ZnOH derivative precludes such an investigation at this time. For this reason, we turned our attention to the well characterized tris(pyrazolyl)hydroborato zinc hydroxide system, with the notion that the observed *trend* in the thermodynamics of these zinc hydroxide/alkoxide interconversions would be related to that in a [NSS] environment.

Scheme 1



(Ar = *p*-$C_6H_4NO_2$, R = Et, Pr^i)

Significantly, we have discovered that $[Tp^{Bu^t,Me}]ZnOH$[3] does indeed react with EtOH to generate $[Tp^{Bu^t,Me}]ZnOEt$; thus, the ethoxide complex $[Tp^{Bu^t,Me}]ZnOEt$ is observed by ^1H NMR spectroscopy to be a significant component in the equilibrium mixture obtained by the reaction of $[Tp^{Bu^t,Me}]ZnOH$ with ethanol-d_6 (Scheme 1). Likewise, solutions of $[Tp^{Bu^t,Me}]ZnOH$ in methanol-d_4 and 2-propanol-d_8 generate equilibrium concentrations of the corresponding alkoxides, $[Tp^{Bu^t,Me}]ZnOMe$ and $[Tp^{Bu^t,Me}]ZnOPr^i$. The formation of these alkoxide complexes is especially noteworthy since Vahrenkamp recently reported that the tris(pyrazolyl)hydroborato zinc complex $[Tp^{Cum,Me}]ZnOH$ is unreactive toward aliphatic as well as benzyl alcohols; nevertheless, Vahrenkamp did demonstrate that *activated* alcohols of sufficient acidity, such as phenols and trifluoroethanol, were capable of reacting with $[Tp^{Cum,Me}]ZnOH$ to give alkoxide derivatives, $[Tp^{Cum,Me}]ZnOR$.[9−11]

Although the equilibrium concentrations of $[Tp^{Bu^t,Me}]ZnOR$ (R = Me, Et, Pr^i) in the aforementioned solutions are such that their

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(2) A second zinc center of each subunit, which is coordinated tetrahedrally to four cysteine residues, is believed to play only a structural role. For a discussion of the structural role of zinc in proteins, see: Meiβner, A.; Haehnel, W.; Vahrenkamp, H. *Chem. Eur. J.* **1997**, *3*, 261−267.

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(10) Also see: Hikichi, S.; Tanaka, M.; Moro-oka, Y.; Kitajima, N. *J. Chem. Soc., Chem. Commun.* **1992**, 814−815.

(11) For $[Tp^{Cum,Me}]ZnSR$ thiolate derivatives, see: Burth, R.; Vahrenkamp, H. *Z. Anorg. Allg. Chem.* **1998**, *624*, 381−385.

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Table 1. Equilibrium Constant and Thermodynamic Data for Alcoholysis Reactions

R	K_R (300 K)[a]	ΔH_R[a]/ kcal mol^{-1}	pK_a (ROH)[b]	heterolytic D(RO−H)[c]/ kcal mol^{-1}	relative heterolytic D(Zn−OR)]/ kcal mol^{-1} [d]
H	1	0	15.74	390.7	0
Me	$1.4(2) \times 10^{-3}$	1.2(1)	15.09	381.7	−10.2
Et	$9(2) \times 10^{-4}$	1.5(2)	15.93	378.6	−13.6
Pr^i	$3(1) \times 10^{-5}$	3.5(2)	17.1	376.7	−17.5
Bu^t	$\approx 10^{-8}$	≈ 8	19.2	375.9	≈ -23

[a] K_{Me} was measured in MeOH. The temperature dependence of K_{Me} over the range 200−300 K gave $\Delta H = 1.2(1)$ kcal mol^{-1} and $\Delta S = -9(1)$ eu. K_R (R = Et, Pr^i, Bu^t) were determined with respect to K_{Me} by measurement of alkoxide exchange equilibria in benzene solution; in view of the low value of K_{Me} it was not possible to measure such a value by equilibration of the hydroxide with MeOH in benzene solution. ΔH_R is calculated with the approximation that $\Delta S \approx 0$ for the alkoxide exchange reactions. The latter approximation was verified experimentally for the zinc methoxide/ethoxide exchange equilibrium [$\Delta S = -0.8(10)$] over the temperature range 250−330 K. Furthermore, other researchers have noted that entropy contributions to equilibria of this type are negligible. See, for example: Bryndza, H. E.; Fong, L. K.; Paciello, R. A.; Tam, W.; Bercaw, J. E. *J. Am. Chem. Soc.* **1987**, *109*, 1444−1456. [b] Data taken from: Murto, J. *Acta Chem. Scand.* **1964**, *18*, 1043−1053. [c] Gas-phase values, taken from Berkowitz, J.; Ellison, G. B.; Gutman, D. *J. Phys. Chem.* **1994**, *98*, 2744−2765 (for H_2O) and Ervin, K. M.; Gronert, S.; Barlow, S. E.; Gilles, M. K.; Harrison, A. G.; Bierbaum, V. M.; DePuy, C. H.; Lineberger, W. C.; Ellison, G. B. *J. Am. Chem. Soc.* **1990**, *112*, 5750−5759 (for ROH). [d] [D(Zn−OR) − D(Zn−OH)] = [D(RO−H) − D(HO−H)] − ΔH.

isolation is prohibitive, they may, nevertheless, be isolated in the absence of water via the reaction of the hydride complex [Tp$^{Bu^t,Me}$]ZnH[12] with the respective alcohol (Scheme 1), and the molecular structure of the ethoxide complex has been determined by X-ray diffraction.[12,13] As noted by Vahrenkamp,[9a] structurally characterized terminal alkoxide derivatives of zinc are rare, but the Zn−O bond length of 1.826(2) Å is comparable to the bond lengths in the few complexes that are known (ca. 1.8−1.90 Å).[9a,14]

The formation of these simple alkoxide derivatives [Tp$^{Bu^t,Me}$]ZnOR is of particular importance because it has been questioned whether the four-coordinate Zn^{2+} center in LADH is capable of lowering the pK_a of an alcohol from ca. 16 to ca. 6, so that it is capable of being deprotonated upon coordination.[15,16] For this reason, we have quantitatively studied the equilibria between [Tp$^{Bu^t,Me}$]ZnOH and ROH (equation 1) by ^{1}H NMR spectroscopy, as summarized in Table 1.[17]

$$[\mathrm{Tp}^{\mathrm{Bu^t,Me}}]\mathrm{ZnOH} + \mathrm{ROH} \overset{K}{\rightleftharpoons} [\mathrm{Tp}^{\mathrm{Bu^t,Me}}]\mathrm{ZnOR} + \mathrm{H_2O} \quad (1)$$

The equilibrium constant (K) for alcoholysis of the zinc hydroxide complex [Tp$^{Bu^t,Me}$]ZnOH is related to the K_a of the alcohol via the expression

$$K = \frac{K_{\mathrm{Zn-OH}}}{K_{\mathrm{Zn-OR}}} \cdot \frac{K_a(\mathrm{ROH})}{K_a(\mathrm{H_2O})}$$

where K_{ZnOH} and K_{ZnOR} are the heterolytic dissociation constants for the Zn−OH and Zn−OR bonds.[18] As such, a knowledge of the relative binding strengths of anionic OH^- and OR^- ligands to zinc is most important *vis-à-vis* the mechanism of action of LADH, but such information is scarce and is principally limited to theoretical calculations.[19] It is, therefore, significant that the temperature dependence of K has allowed us to determine that the Zn−OH heterolytic bond dissociation energy is 10.2 kcal mol^{-1} greater than that of the Zn−OMe bond energy (Table 1), a value that is significantly less than that predicted for $[Zn(OH)]^+$ versus $[Zn(OMe)]^+$ (15.6 kcal mol^{-1}).[19a] The data in Table 1 also indicate that the heterolytic Zn−OR bond dissociation energy decreases rapidly upon increasing the bulk of R. More importantly, the heterolytic Zn−OR bond dissociation energy is more sensitive to the nature of R than is the heterolytic H−OR bond dissociation energy (Table 1),[20] with the result that the alcoholysis equilibrium constants decrease across the series MeOH > EtOH > Pr^{i}OH > Bu^{t}OH. Notably, although this trend is not predicted on the basis of the gas-phase acidities of ROH, such a trend is consistent with the pK_a values of the alcohols in aqueous solution.

Another important issue surrounding the proposed role of alkoxide intermediates is concerned with whether they are sufficiently activated to be capable of transferring a "hydride" to a substrate. Compelling evidence which indicates that the alkoxide complexes [Tp$^{Bu^t,Me}$]ZnOR (R = Et, Pr^i) are indeed capable of such a transformation is provided by studies employing NAD$^+$ hydride acceptor mimics, such as *p*-nitrobenzaldehyde.[9b,21] For example, [Tp$^{Bu^t,Me}$]ZnOEt reacts with ArCHO (Ar = *p*-$C_6H_4NO_2$) in benzene to yield [Tp$^{Bu^t,Me}$]ZnOCH$_2$Ar and MeCHO.[22] Furthermore, solutions of ArCHO in ROH (R = Me, Et, Pr^i) yield ArCH$_2$OH at ca. 90 °C in the presence of [Tp$^{Bu^t,Me}$]ZnOH.

In summary, studies on well-defined structurally characterized four-coordinate zinc hydroxide and alkoxide complexes provide strong support for some of the critical steps proposed for the mechanism of action of LADH. Specifically, (i) four-coordinate zinc alkoxide derivatives [Tp$^{Bu^t,Me}$]ZnOR are indeed generated upon reaction of the hydroxide complex [Tp$^{Bu^t,Me}$]ZnOH with aliphatic alcohols and (ii) the alkoxide functionality in [Tp$^{Bu^t,Me}$]ZnOR is activated toward "hydride" transfer.

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Supporting Information Available: Experimental and crystallographic information (25 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) See Supporting Information.

(13) [Tp$^{Bu^t,Me}$]ZnOMe and [Tp$^{Bu^t,Me}$]ZnOEt have previously been reported to be generated by vacuum pyrolysis of [Tp$^{Bu^t,Me}$]ZnOCO$_2$R, but recrystallization attempts yielded the hydroxide [Tp$^{Bu^t,Me}$]ZnOH. See: (a) Ruf, M.; Schell, F. A.; Walz, R.; Vahrenkamp, H. *Chem. Ber./Recueil* **1997**, *130*, 101−104. (b) Alsfasser, R.; Ruf, M.; Trofimenko, S.; Vahrenkamp, H. *Chem. Ber.* **1993**, *126*, 703−710.

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(16) For recent studies on the determination of pK_a of values of alcohols in ternary complexes, see: Pocker, Y.; Page, J. D. *J. Biol. Chem.* **1990**, *265*, 22101−22108.

(17) The data presented in Table 1 indicate that the equilibria lie strongly in favor of the hydroxide. Such an observation does not preclude an alkoxide species as a viable intermediate of the LADH catalytic cycle, since it is the rate constant for its formation that determines its ability to be an active intermediate.

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(19) See, for example: (a) Garmer, D. R.; Gresh, N. *J. Am. Chem. Soc.* **1994**, *116*, 3556−3567. (b) Gresh, N. *J. Compu. Chem.* **1995**, *16*, 856−882.

(20) The greater sensitivity of the Zn−OR bond dissociation energy is presumably due to the greater role that steric interactions play in the zinc complex than in the alcohol.

(21) Kimura, E.; Shionoya, M.; Hoshino, A.; Ikeda, T.; Yamada, Y. *J. Am. Chem. Soc.* **1992**, *114*, 10134−10137.

(22) [Tp$^{Bu^t,Me}$]ZnOCH$_2$Ar is more readily obtained *via* the reaction of [Tp$^{Bu^t,Me}$]ZnH with ArCH$_2$OH. Although [Tp$^{Bu^t,Me}$]ZnOCH$_2$Ar does not correspond directly to one of the catalytic intermediates illustrated in Scheme 1, since it is a result of the reduced product (ArCH$_2$OH) binding to the zinc center, it does, nevertheless provide clear evidence for zinc-mediated hydride transfer from ROH to ArCHO.